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Aneuploidy in stem cells

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Abstract

Stem cells hold enormous promise for regenerative

medicine as well as for engineering of model systems to study diseases and develop new drugs. The discovery of protocols that allow for generating induced pluripotent stem cells (iPSCs) from somatic cells has brought this promise steps closer to reality. However, as somatic cells might have accumulated various chromosomal abnormalities, including aneuploidies throughout their lives, the resulting iPSCs might no longer carry the perfect blueprint for the tissue to be generated, or worse, become at risk of adopting a malignant fate. In this review, we discuss the contribution of aneuploidy to healthy tissues and how aneuploidy can lead to disease. Furthermore, we review the differences between how somatic cells and stem cells respond to aneuploidy.

Key words: Chromosomal instability; Aneuploidy; Embryonic stem cells; Induced pluripotent stem cells; Mesenchymal stem cells

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Core tip: Stem cells hold great therapeutic promise for regenerative medicine, especially with new protocols that can create induced pluripotent stem cells from terminally differentiated cells. However, somatic cells and stem cells cope differently with genomic instability. Therefore, it will be of the utmost importance to assess genomic integrity when preparing stem cell cultures for future therapy.

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INTRODUCTION

Chromosomal instability (CIN) is a process that leads to cells with unbalanced genomes, containing structural

abnormalities (*i.e.*, structural CIN leading to amplification, deletions, translocations), numerical abnormalities (*i.e.*, numerical CIN), or both. Numerical CIN leads to aneuploidy, a state in which cells have abnormal numbers of whole chromosomes. While the majority of all cancers are aneuploid^[1], aneuploidy itself appears to act anti-proliferative in non-transformed cells, suggesting that cancer cells somehow have adjusted to the adverse effects of aneuploidy^[2,3]. Emerging evidence is indicating that aneuploidy in somatic cells increases with age and might even contribute to natural ageing^[4-8]. In fact, we are only beginning to understand how our somatic cells and stem cells cope with loss of genomic integrity. As future regenerative medicine-based therapies will likely make use of induced pluripotent stem cells (iPSCs) derived from somatic (aged) patient's cells, aneuploidy will become an important parameter to test for when assessing the quality of the transplanted cells. Furthermore, much is still unknown regarding the effects aneuploidy on the functionality of both somatic stem cells and iPSCs. In this review, we will discuss the impact of aneuploidy on healthy stem cells, how aneuploidy can lead to disease, and how stem cells cope with genomic instability.

ANEUPLOIDY IN HEALTHY TISSUES

Even though systemic aneuploidies or high CIN rates are poorly tolerated during development, various lines of evidence indicate that some of our tissues can cope remarkably well with aneuploidy^[9] and even show aneuploidy under "normal" conditions^[4]. Two tissues that have been associated with increased aneuploidy frequencies are brain and liver.

Aneuploidy in the brain

Aneuploidy in mature brain can affect both the neuronal and non-neuronal populations of the cortex^[10,11], and is thought to originate in ventricular zone progenitors that encounter a variety of cell division defects during embryogenesis^[12,13]. While the most common aneuploid event in progenitors is the loss of a single chromosome, progenitors with up to 5 chromosome losses have been reported. Based on this and other work, cumulatively, 10% of neurons in a healthy brain are estimated to be aneuploid^[14], but the role and fate of these cells remains unclear. For more extensive review see Bushman *et al.*^[15].

The precise contribution of aneuploidy to the healthy human brain remains unclear. One possible explanation is that aneuploidy actually serves a function and is part of a "normal" process during brain development resulting in heterogeneous neuronal and glial populations that contribute to the wide functions that neurons can have^[16]. An alternative explanation however is that aneuploidy rates have been overestimated due to technical limitations of the protocols used to quantify aneuploidy in neurons (in most cases interphase FISH)^[17], which was reinforced by recent studies that used single cell next generation sequencing to determine chromosome

copy numbers in adult brains^[14,18-20]. Aneuploidy in the brain has furthermore been linked to various pathologies such as Alzheimer's disease (AD) (increased trisomy of chromosome 21), schizophrenia, and autism^[21,22]. Further quantification of aneuploidy in the (diseased) human brain is needed to resolve these conflicting observations.

Aneuploidy in the liver

Roughly half of the hepatocytes in an adult liver are polyploid or aneuploid^[23]. Interestingly, liver regeneration has been attributed to proliferation of mature hepatocytes, but not liver stem cells^[24]. This is in line with other observations that stem cells tolerate aneuploidy poorly^[4,9]. The aneuploidization and polyploidization of hepatocytes has been suggested to contribute to a great variety of hepatocyte genotypes that could help the liver adapt to different insults and chronic stressors^[23]. While single cell next generation sequencing failed to detect aneuploidy in adult neurons, it did confirm the polyploidy and aneuploidy rates previously measured in hepatocytes^[18], further emphasizing the importance of increased efforts to quantify aneuploidy in various tissues using several techniques.

ANEUPLOIDY IN HUMAN PATHOLOGIES

Stem cells and ageing

Functional exhaustion of adult stem cells is an important contributing factor to natural ageing, as stem cells are essential for tissue maintenance, especially in tissues that have a high turnover rate, such as the intestinal wall and the skin^[25]. Indeed, ageing coincides with a functional decline of stem cell function in various organs, even though this is not always accompanied by reduced stem cell numbers. For example, in humans and some mouse strains the number of hematopoietic stem cells even increases during ageing^[26-28], but their potential to differentiate decreases^[28]. However, whether increased aneuploidy rates as observed in ageing tissues^[8,29] contribute to stem cells exhaustion needs further testing. There are several syndromes that are caused by systemic aneuploidy that exhibit also premature ageing features, such as Down's syndrome (DS) or Edward's syndrome. However, how the systemic aneuploidy in these patients impacts stem cell integrity and stem cell numbers requires further investigation.

Stable systemic aneuploidy: DS

The most well known condition linked to systemic aneuploidy is DS. This syndrome, caused by a systemic gain of chromosome 21 (trisomy 21), is associated with developmental and cognitive defects and affects about 1 in 700 individuals^[30]. In line with a role of aneuploidy in natural ageing^[8], DS is associated with an earlier onset of aging-related pathologies such as AD, and an increased incidence of cancer^[31]. One proposed driver of the accelerated ageing phenotype in DS is the observed overexpression of *CDKN2A*, as a result of

epigenetic remodulation in various cell lineages including hematopoietic stem cells, mammary epithelium, fibroblasts and neural progenitors cells^[32]. Enhanced *CDKN2A* expression is associated with senescence and stem cell self-renewal defects, further emphasizing the link between aneuploidy and ageing.

STEM CELLS AND CIN

Stem cell biology is a rapidly developing field recently revolutionized through the discovery for protocols to revert terminally differentiated cells back into a pluripotent state (iPSCs)^[33]. Stem cells hold great therapeutic promise for the treatment of a large number of diseases and are defined as cell lineages that have 3 cardinal features: (1) self-renewal through asymmetric cell division yielding one stem cell and one differentiated cell or symmetric division yielding two stem cells; (2) the capacity to produce multiple cell lineages; and (3) the potential to proliferate extensively^[34,35]. We are only beginning to understand how to isolate and maintain stem cells in tissue culture and how to differentiate them into specific tissues, all of which are crucially important to exploit stem cells in future therapies^[36].

Stem cell potency

Stem cells can be subdivided in two subtypes that differ in their differentiation potential or potency: (1) Pluripotent stem cells: Embryonic stem cells (ESCs) are pluripotent, which means that they can form any embryonic tissue, embryonic therefore can form a complete and viable organism when injected into a blastocyst. Mouse ESC culture has revolutionized biology as it allowed for making transgenic and knockout mice^[37,38]; Pluripotent stem cells can form any of the three germ layers of an embryo. The recent discovery of protocols to induce pluripotency in differentiated cells, yielding iPSCs^[33] has propelled the stem cell field as a whole and made this stem cell subtype the current central tool in stem cell research, as, in theory, iPSC protocols would allow us to make any cell type from any patient's somatic cell, overcoming graft versus host disease and omitting the ethical concerns of human ESCs; and (2) The second subtype of stem cells are multipotent, somatic or adult stem cells. These stem cells that can form a number of lineages, typically within one tissue type. Examples of somatic stem cells are neural stem cells, mammary stem cells or hematopoietic stem cells. In many cases somatic stem cells produce unipotent proliferative cells or transit amplifying cells, *e.g.*, through asymmetric stem cell division. These cells can still replicate, but only form one cell lineage before cells terminally differentiate and exit the cell cycle^[39] and are therefore not considered to be genuine stem cells.

HOW CAN STEM CELLS RESPOND TO ANEUPLOIDY?

Because of their important role in development and

tissue maintenance, stem cells have to safeguard their genome integrity. Any genetic alteration that may occur in a dividing stem cell will be inherited by the entire lineage emerging from this single stem cell and thus give rise to severe developmental defects or pathologies. Genomic integrity maintenance in stem cells is not only important for proper embryonic development and adult tissue homeostasis, but also an essential requirement for the use of stem cells in regenerative medicine and research. This is particularly true for mesenchymal stem cells (MSCs), that are already used in therapy. Importantly, both embryonic and somatic stem cells have developed ways to prevent the negative effects of mutations and aneuploidies resulting in an increased DNA damage response in stem cells as compared to somatic cells, thus preventing structural CIN^[40].

DNA damage repair systems in stem cells

One way stem cells accomplish an increased DNA damage response is by increasing the expression of genes involved in DNA repair resulting in increased efficiency in repairing DNA lesions, when compared to differentiated cells^[40]. The most dangerous form of DNA damage is the formation of DNA double strand breaks (DSBs). These can arise from replication stress, reactive oxygen species (ROS), mutagens and other DNA damaging events. To repair DSBs, cells employ two main DNA repair systems: Non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ is an error-prone form of repair taking place in the G1 phase of the cell cycle in which the sequence flanking the break point is resected followed by blunt end ligation of the DNA ends, which by definition results in small deletions in the DNA sequence. In contrast, HR utilizes the sister chromatid as a repair template to fully repair the DSB, and can therefore only occur when sister chromatids are available, in the G2 phase of the cell cycle. Another way by which stem cells maintain genomic fidelity is by their predominant use of HR to repair DSBs, while differentiated (non-proliferating) cells can only use NHEJ, as the latter have exited the cell cycle and therefore have no "access" to duplicated sister chromatids^[41,42]. Therefore, the preferred use of HR to repair DNA DSBs combined with more sensitive DNA damage signaling helps stem cells to maintain genomic integrity.

The safe way out: Apoptosis and differentiation

An alternative method to prevent daughter cells from inheriting genomic aberrations is by eliminating the aberrant stem cells from the stem cell pool. This can be done through apoptosis, removing the cell altogether, or by differentiating the compromised stem cell in order to avoid further cell divisions. Indeed, when encountering DNA damage, stem cells activate the p53 pathway to mobilize the DNA repair machinery. When damage remains unrepaired, p53 activity promotes apoptosis. While apoptosis is the main response to DNA damage in stem cells, induction of p53 in human stem cells can

Table 1 Reported chromosomal aberrations in different stem cell types

| Species | Stem cell type | Chromosomal aberration |
|---------|-------------------------------|------------------------|
| Human | Cultured embryonic stem cells | +1, +12, +14, +17, +20 |
| | Mesenchymal stem cells | 4N, -13 |
| Mouse | Cultured embryonic stem cells | +4, +5, +10, +X |
| | | +8 |

also result in spontaneous differentiation^[43], however, which factors determine the switch between these two choices are so far unclear.

This is exemplified further by the ways by which different stem cell types respond to DNA damage. For example, hematopoietic stem cells (HSCs) enhance DNA repair and stem cell maintenance, and prevent apoptosis to avoid depletion of the HSC pool. Intestinal stem cells on the other hand favor increased cell death and melanocytes respond by terminal differentiation^[44]. Therefore, different responses to DNA damage in different stem cell populations will yield different effects on the integrity of the stem cell pool and their downstream potencies.

STEM CELLS COPE POORLY WITH NUMERICAL CIN

Numerical CIN and the resulting aneuploidy is detrimental for embryonic development, evidenced by early embryonic death observed in mouse models in which high grade numerical CIN was provoked systemically, see for extensive review^[45-47]. While these observations led to the prevailing view that high grade numerical CIN is never tolerated, more recent observations are nuancing this view. For instance, when numerical CIN was provoked in a tissue specific fashion, in mouse epidermis, epidermal hair follicle stem cells were rapidly depleted, while the more committed transit amplifying (unipotent) cells tolerated the resulting high grade aneuploidy remarkably well^[9]. Furthermore, while aneuploid cells seem to accumulate in various somatic cell types in the ageing mouse, aneuploidy in stem cell lineages in the same mice remains rare, further indicating that stem cells are well protected against (or ultra sensitive to) numerical chromosome abnormalities^[4]. However, even though these observations are suggestive of an aneuploidy checkpoint in stem cells, more work is needed to reveal if this checkpoint does exist and if so, how this checkpoint operates.

DO CULTURE CONDITIONS IMPACT STEM CELL GENOMIC INTEGRITY?

As stem cells are typically isolated in small quantities and iPSC protocols are still quite inefficient, stem cells are exposed to a period of tissue culture stress that can yield (further) CIN. While the majority of such alterations will be negatively selected and therefore disappear within a few passages, some genetic alterations could result in

proliferative advantages and outcompete the “normal” stem cells, a process known as “culture adaptation”^[48] resulting in late passage cultures that grow better and show better plating efficiencies^[49].

Frequently reported chromosome aberrations in stem cells

Various cytogenetic abnormalities have been reported in cultured ESCs, with varying frequencies in different cell strains. The most common abnormality reported in murine ESCs is trisomy of chromosome 8 (Table 1). Trisomies of chromosome 12, 14 and 17 are most frequently observed in human ESCs^[50], but not necessarily within the same ES cell line. Further studies that investigated the genomic integrity of > 150 human ES cell lines and 220 iPSC lines found that, while the majority of the stem cell lines were euploid, approximately 12%-13% of human ES cell lines and similar fractions of iPSC lines showed whole chromosome abnormalities^[49,51].

Notably in ESC culture, chromosomal abnormalities increased with prolonged culturing as late passage cultures had approximately twice as many aberrations, which typically involved gain of chromosomes^[49]. In line with culture adaptation, chromosome aberrations were non-random with 60% of the aneuploid stem cell cultures showing abnormalities for chromosomes 1, 12, 17 and/or 20 (Table 1)^[49]. Chromosome 12 gain has been observed by others as well^[52,53] and although selection for chromosome 12 is likely to be driven by multiple genes, it is tempting to speculate that *NANOG* is a key driver for this commonly observed trisomy in stem cell cultures, possibly together with the pluripotency related *DPPA3* and *GDF3*, and the cell cycle regulator *CCND2*. Furthermore, the oncogene *KRAS* is also located on chromosome 12^[49,54]. Importantly, culture conditions appear to also have an important impact on genomic integrity of stem cell cultures, for instance the use of fetal bovine serum as growth supplement results in increased chromosomal abnormalities^[55]. Last, but certainly not least important, iPSC cells are typically derived from somatic (differentiated cells) that appear to tolerate aneuploidy much better than previously anticipated^[9] and therefore the founder cells could already have been aneuploid to start out with. Aneuploidies in these founder cells might lower iPSC protocol's efficiency if the induced stem cells do not tolerate the aneuploidies. Even worse, when aneuploidies in the founder cells are subtle, the aneuploidies could be maintained in the resulting iPSC clones.

As MSCs are frequently used in therapy, their genomic stability is routinely assessed. The most commonly reported type of aberration in MSCs several passages after cell expansion is tetraploidization, observed in 6 out of 21 patient-derived cell lines^[56]. Tetraploidy was observed only in a minority of examined metaphases (approximately 1 in 20-30 per cell line), and whether the contribution of these tetraploid cells will have significant consequences *in vivo* needs further investigation. Other reported aberrations include a preferential loss of chromosome 13 in later passages of the human MSC line UE6E7T-3 (Table 1)^[57],

and the emergence of clonal trisomies for chromosomes 4, 5, 10, and X in a male patient-derived MSC^[58]. Therefore, while cytogenetic aberrations are widely reported for MSCs, their ultimate effect on MSC proliferation and potency *in vivo* are less well understood. This also holds true for other stem cell populations *in vivo*. Novel karyotyping methods (for extensive review see Bakker *et al.*^[17]) that circumvent the limitations of existing techniques will be instrumental in resolving both the incidence and effects of aneuploidy in adult stem cells in various different tissues.

CONCLUSION

Stem cells are the origin of tissue homeostasis and therefore crucially rely on genomic integrity. Fortunately, stem cells appear to be much more sensitive to DNA damage and aneuploidy than their differentiated counterparts^[9,40-42]. However, as iPSC protocols force terminally differentiated cells back into the cell cycle, cells might accumulate mutations in the process of becoming pluripotent, or even harbor chromosomal abnormalities before dedifferentiation starts. Therefore, as iPSC protocols are becoming the new standard in regenerative medicine, it will be of the utmost importance to develop sequencing pipelines that can ensure chromosomal fidelity of the engineered iPSCs or iPSC-derived tissues.

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